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SCIENCE

FRIDAY, NOVEMBER 12, 1920

THE PROTEINS AND COLLOID CHEMISTRY¹

CONTENTS

<i>The Proteins and Colloid Chemistry:</i> DR. JACQUES LOEB	449
<i>The African Rift Valleys:</i> PROFESSOR W. M. DAVIS	456
<i>Scientific Events:—</i>	
<i>The Sixteenth Annual New England Intercollegiate Geological Excursion; "Physiological Reviews;" University of Pennsylvania Lectures; The Section of Zoology of the American Association</i>	458
<i>Scientific Notes and News</i>	461
<i>University and Educational News</i>	464
<i>Discussion and Correspondence:—</i>	
<i>The Preservation of Wild Life:</i> PROFESSOR V. E. SHELFORD. <i>Predilection and Sampling of Human Heights:</i> PROFESSOR EDWIN G. BORING. <i>The Plight of Scientific Periodicals:</i> DR. HOWARD S. REED. <i>Road Reflections:</i> H. H. PLATT. <i>The Influence of Fresh Food in Lactation:</i> E. B. FORBES. <i>Swarming of Anopheles:</i> DR. L. O. HOWARD.....	465
<i>The Works of Ameghino:</i> PROFESSOR ROY L. MOODIE	469
<i>Special Articles:—</i>	
<i>The Free Martin and its Reciprocal; Oposum, Man, Dog:</i> PROFESSOR CARL G. HARTMAN	469
<i>The Nebraska Academy of Sciences:</i> C. O. CARLSON	471

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I

THE proteins, like certain other constituents of protoplasm, are colloidal in character, *i. e.*, they are not able to diffuse through animal membranes which are permeable to crystalloids. For this reason a number of authors have tried to explain the behavior of proteins from the viewpoint of the newer concepts of colloid chemistry. Foremost among these concepts is the idea that the reactions between colloids and other bodies are not determined by the purely chemical forces of primary or secondary valency but follow the rules of "adsorption." Although a number of authors, during the last twenty years, *e. g.*, Bugarszky and Liebermann, Hardy, Pauli, Robertson, Sørensen, and others, have advocated a chemical conception of the reactions of proteins, their experiments failed to convince the other side since these experiments could just as well be explained on the basis of the adsorption theory. There were two reasons for this failure. First, the experiments did not show that ions combined with proteins in the typical ratio in which the same ions combine with crystalloids. This proof only became possible when it was recognized that the hydrogen ion concentration of the protein solution determines the amount of ion entering into combination with a protein, and that therefore the ratios in which different ions combine with proteins must be compared for the same hydrogen ion concentrations. Since the former workers were in the habit of comparing the effects of

¹ Address delivered before the Harvey Society, October 16, 1920. The writer's experiments, on which this address is based, have appeared in the *J. Gen. Physiol.*, 1918-19, I., 39, 237, 363, 483, 559; 1919-20, II., 87; 1920-21, III., 85.

the same quantities of acid or alkali added instead of comparing the behavior of proteins at the same hydrogen ion concentration they were not able to furnish the final proof for the purely chemical character of the combinations between ions and proteins, and nothing prevented chemists from assuming that proteins formed only adsorption compounds with acids, bases, and neutral salts.

The second reason for the failure to prove the purely chemical character of the protein compounds lay in the so-called Hofmeister series of ion effects. Hofmeister was the first to investigate the effects of different salts on the physical properties of proteins, and he and his followers observed that the relative effects of anions on the precipitation, the swelling, and other properties of proteins was very definite and that the anions could be arranged in definite series according to their relative efficiency, the order being independent of the nature of the cation. Similar series were also found for the cations, though these series seemed to be less definite. These Hofmeister series were a puzzle inasmuch as it was impossible to discover in them any relation to the typical combining ratios of the ions, and this lack of chemical character in the Hofmeister series induced chemists to explain these series on the assumption of a selective adsorption of these ions by the colloids.

To illustrate this we will quote the order which, according to Pauli, represents the relative efficiency of different acids on the viscosity of blood albumin,

HCl > monochloroacetic > oxalic > dichloroacetic > citric > acetic > sulfuric > trichloroacetic acid,

where HCl increased the viscosity most and trichloroacetic or sulfuric least. In this series the strong monobasic acid HCl is followed by the weak monochloroacetic acid, this is followed by the dibasic oxalic acid; later follows a weak tribasic citric acid, then the very weak monobasic acetic acid, then the strong dibasic sulfuric acid, and finally again a monobasic acid, trichloroacetic. Pauli is a believer in the chemical theory of the be-

havior of proteins but it is impossible to harmonize his series of anions with any purely chemical theory of the behavior of proteins.

The ion series of Hofmeister are no more favorable for a chemical conception. Thus, according to Hofmeister, gelatin swells more in chlorides, bromides and nitrates than in water, while in acetates, tartrates, citrates, or sugar it swells less than in water. R. Lillie arranges ions according to their depressing effect on the osmotic pressure of gelatin solution in the following way,

$\text{Cl} > \text{SO}_4 > \text{NO}_3 > \text{Br} > \text{I} > \text{CNS}$.

These series again betray no relation to the stoichiometrical properties of the ions. As long as these Hofmeister ion series were believed to have a real existence it seemed futile to decide for or against a purely chemical theory of the behavior of colloids since even with a bias in favor of a chemical theory the Hofmeister series remained a puzzle.

The writer believes to have removed these difficulties by using protein solutions of the same hydrogen ion concentration as the standard of comparison. In this way he was able to show that acids, alkalies, and neutral salts combine with proteins by the same chemical forces of primary valency by which they combine with crystalloids, and that, moreover, the influence of the different ions upon the physical properties of proteins can be predicted from the general combining ratios of these ions. The so-called Hofmeister series have no real existence, being the result of the fact that the older workers failed to measure the most important variable in the case, namely the hydrogen ion concentration of their protein solutions, a failure for which they can not be blamed since the methods were not sufficiently developed.

II

Pauli and a number of other workers assume that both ions of a neutral salt are adsorbed simultaneously by non-ionized protein molecules. If we consider the hydrogen ion concentration of the proteins we can show

that only the cation or only the anion or that neither ion can combine at one time with a protein; and that it depends solely on the hydrogen ion concentration of the solution which of the three possibilities exists.

Proteins exist in three states, defined by their hydrogen ion concentration, namely, (a) as non-ionogenic or isoelectric protein, (b) metal proteinate (*e. g.*, Na or Ca proteinate), and (c) protein-acid salts (*e. g.*, protein ehloride, protein sulfate, etc.). We will use gelatin as an illustration. At one definite hydrogen ion concentration, namely $10^{-4.7}$ N (or in Sørensen's logarithmic symbol at $\text{pH}=4.7$), gelatin can combine practically with neither anion nor cation of an electrolyte. At a $\text{pH} > 4.7$ it can combine only with cations (forming metal gelatinate, *e. g.*, Na gelatinate), at a $\text{pH} < 4.7$ it combines with anions (forming gelatin chloride, etc.). This was proved in the following way: Doses of 1 gm. of finely powdered commercial gelatin (going through sieve 60 but not through 80), which happened to have a pH of 7.0, were brought to a different hydrogen ion concentration by putting them for 1 hour at about 15°C . into 100 c.c. of HNO_3 solutions varying in concentration from M/8192 to M/8. After this they were put on a filter, the acid being allowed to drain off, and were washed once or twice with 25 c.c. of cold water (of 5°C . or less) to remove remnants of the acid between the granules of the powdered gelatin. These different doses of 1 gm. of gelatin now possessing a different pH were all put for 1 hour into beakers containing the same concentration, *e. g.*, M/64, of silver nitrate at a temperature of 15°C . They were then put on a filter and washed 6 or 8 times each with 25 c.c. of ice cold water; the wash water must be cold since otherwise the particles will coalesce and the washing will be incomplete. This washing serves the purpose of removing the AgNO_3 held in solution between the granules, thus allowing us to ascertain where the Ag is in combination with gelatin and where it is not in combination, since the Ag not in combination with gelatin can be removed by the washing while the former can

not, or at least only extremely slowly by altering the pH . After having removed the AgNO_3 not in combination with gelatin by washing with ice cold water we melt the gelatin by heating to 40°C ., adding enough distilled water to bring the volume of each gelatin solution to 100 c.c., determine the pH of each solution potentiometrically or colorimetrically, and expose the solutions in test-tubes to light, the previous manipulations having been carried out in a dark room (with the exception of the determination of pH , for which only part of the gelatin solution was used). In 20 minutes all the gelatin solutions with a $\text{pH} > 4.7$, *i. e.*, from pH 4.8 and above, become opaque and then black, while all the solutions of $\text{pH} < 4.7$, *i. e.*, from 4.6 and below, remain transparent even when exposed to light for months or years. The solutions of pH 4.7 become opaque, but remain white, no matter how long they may have been exposed to light. At this pH —the isoelectric point—gelatin is not in combination with Ag, but it is insoluble. Hence the cation Ag is only in chemical combination with gelatin when the pH is > 4.7 . At pH 4.7 or below gelatin is not able to combine with Ag ionogenically. This statement was confirmed by volumetric analysis.

The same tests can be made for any other cation the presence of which can be easily demonstrated. Thus when powdered gelatin of different pH is treated with NiCl_2 and the NiCl_2 not in combination with gelatin be removed by washing with ice cold water, the presence of Ni can be demonstrated in all gelatin solutions with a $\text{pH} > 4.7$ by using dimethylglyoxime as an indicator. All gelatin solutions of pH of 4.8 or above assume a crimson color upon the addition of dimethylglyoxime, while all the others remain colorless. When we treat gelatin with copper acetate, and wash afterwards, the gelatin is blue and opaque when its pH is 4.8 or above, but is colorless and clear for $\text{pH} < 4.7$. Most striking are the results with basic dyes, *e. g.*, basic fuchsin or neutral red, after sufficient washing with cold water; only those gela-

tin solutions are red whose pH is above 4.7, while the others are colorless.

On the acid side of the isoelectric point, *i. e.*, at $\text{pH} < 4.7$, the gelatin is in combination with the anion of the salt used. This can be demonstrated in the same way by bringing different doses of powdered gelatin to different pH and treating them for one hour with a weak solution of a salt whose anion easily betrays itself, *e. g.*, $\text{M}/128 \text{K}_4\text{Fe}(\text{CN})_6$. If after this treatment the powdered gelatin is washed six times with cold water to remove the $\text{Fe}(\text{CN})_6$ not in chemical combination with gelatin and if 1 per cent. solutions of these different samples of gelatin are made, it is found that when the pH is < 4.7 the gelatin solution turns blue after a few days (due to the formation of ferric salt), while solutions of gelatin with a pH of 4.7 or above remain permanently colorless. Hence gelatin enters into chemical combination with the anion $\text{Fe}(\text{CN})_6$ only when pH is < 4.7 . The same can be demonstrated through the addition of ferric salt when gelatin has been treated with NaCNS , the anion CNS being in combination with gelatin only where the pH is < 4.7 . Acid dyes, like acid fuchsin, combine with gelatin only when pH is < 4.7 .

In this way it can be shown that when the pH is > 4.7 gelatin can combine only with cations; when pH is < 4.7 it can combine only with anions, while at pH 4.7 (the isoelectric point) it can combine with neither anion nor cation. The idea that both ions influence a protein simultaneously is no longer tenable.

It follows also that a protein solution is not adequately defined by its concentration of protein but that the hydrogen ion concentration must also be known since each protein occurs in three different forms—possibly isomers—according to its hydrogen ion concentration.

In the experiments just discussed it was necessary to wash the powdered gelatin to find out at which pH an ion was in combination with the gelatin. This has led some authors to the belief that in all my experiments the washing was a necessary part of

the procedure. I therefore will call especial attention to the fact that the experiments to be described in the rest of the paper were carried out with isoelectric gelatin to which just enough acid or alkali was added to bring it to the hydrogen ion concentration required for the purpose of the experiment.

III

When a protein is in a salt solution, *e. g.*, NaCl , it will combine with Na forming sodium proteinate as soon as the pH is higher than the isoelectric point of the protein; when, however, the pH falls below that of the isoelectric point of the protein the Na is given off and protein chloride is formed.

Moreover, the writer has been able to show by volumetric analysis that the quantity of anion or cation in combination with the protein is an unequivocal function of the pH. When we add HCl to isoelectric gelatin and determine the pH we always find the same amount of Cl in combination with a given mass of originally isoelectric gelatin for the same pH; so that if we know the pH and the concentration of originally isoelectric gelatin present we can also tell how much Cl is in combination with the protein for this pH. The same is true when we add an alkali to the isoelectric gelatin. For the same pH the amount of cation in combination is always the same. These facts have led the writer to propose the following theory. When we add an acid, *e. g.*, HCl , to isoelectric gelatin (or any other isoelectric protein) an equilibrium is established between free HCl , protein chloride, and non-ionogenic or isoelectric protein; when we add alkali an equilibrium is established between metal proteinate, non-ionized protein, and the hydrogen ions. Sørensen was led to a similar view on the basis of entirely different experiments.

IV

This fact that the hydrogen ion concentration of a protein solution determines the quantity of protein salt formed is the basis on which the following proof for the purely chemical character of the combination be-

tween proteins and other bodies rests. The experiments mentioned thus far in this paper do not yet allow us to decide whether the ions are "adsorbed" or in chemical combination with the proteins. We will now show that acids and bases combine with proteins

and an alkali. This can be proved in the following way. We know that a weak dibasic or tribasic acid gives off one hydrogen ion more readily than both or all three; while in a strong dibasic acid, like H_2SO_4 , both hydrogen ions are held with a sufficiently small

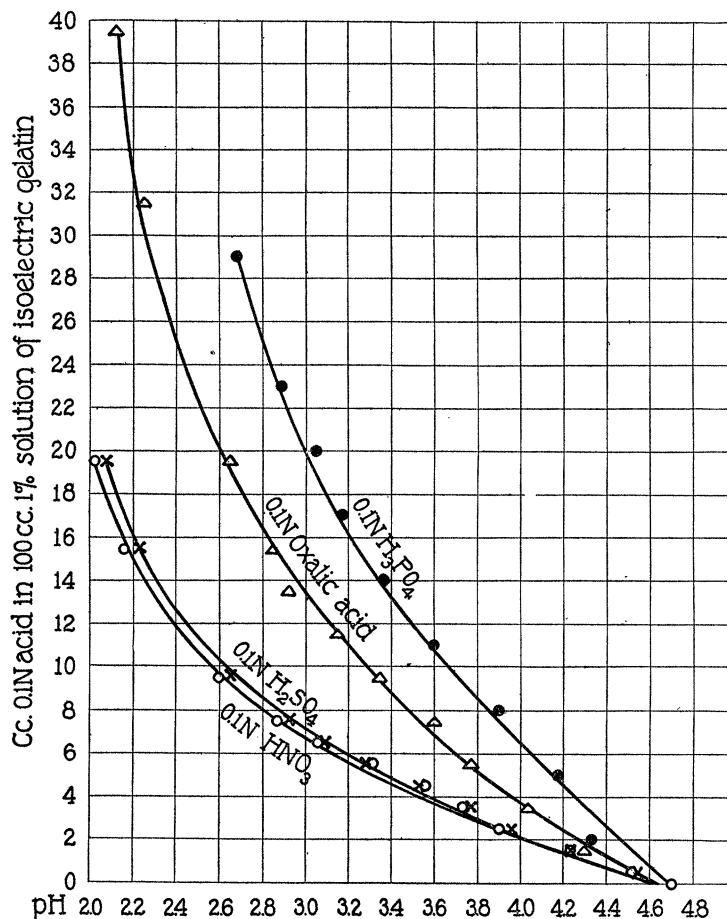


FIG. 1. The ordinates represent the c.c. of 0.1 N acid in 100 c.c. of 1 per cent. solution of isoelectric gelatin required to bring the solution to the pH indicated in the abscissæ. The curves for 0.1 N H_2SO_4 and 0.1 N HNO_3 are identical while the values for H_3PO_4 and oxalic acid differ, being approximately in the ratio of HNO_3 : oxalic acid: H_3PO_4 as 1: 2: 3.

in the same way as they combine with crystalline compounds, namely by the purely chemical forces of primary valency. The combination between acids and proteins is analogous to that between acids and NH_3 , and the combination between bases and proteins is analogous to that between CH_3COOH

electrostatic force to be easily removed. If the forces which determine the reaction between these acids and proteins are purely chemical it would follow that three times as many c.c. of 0.1 NH_3PO_4 are required to bring 100 c.c. of 1 per cent. solution of isoelectric gelatin to a given pH, *e. g.*, 3.0, as are re-

quired in the case of HNO_3 or HCl ; while twice as many c.c. of 0.1 N oxalic as of HNO_3 should be required. On the other hand, it should require just as many c.c. of 0.1 N H_2SO_4 as HNO_3 . Fig. 1 shows that this is the case. The ordinates of this figure are the c.c. of 0.1 N acid required to bring 1 gm. of isoelectric gelatin to the pH indicated in the abscissae by the four acids mentioned, namely HNO_3 , H_2SO_4 , oxalic and phosphoric acids. The curves for H_2SO_4 and HNO_3 are identical while, for the same pH, the value for H_3PO_4 is always approximately three times and the value for oxalic acid is always approximately twice as high as for HNO_3 .

On the basis of the same reasoning as applied to acids we should expect that equal numbers of c.c. of 0.1 N $\text{Ca}(\text{OH})_2$ and $\text{Ba}(\text{OH})_2$ as of LiOH , NaOH , and KOH should be required to bring 100 c.c. of a 1 per cent. solution of isoelectric gelatin to the same pH and the writer was able to show that this is the case. Similar results were obtained with crystalline egg albumin.

When we have a solution of a gelatin-acid salt of originally 1 per cent. isoelectric gelatin and of a certain pH, *e. g.*, 3.0, we have free acid in the solution and a certain amount of the anion of the acid in combination with gelatin. We can find out by volumetric analysis how much of the anion is in combination with the protein by making certain corrections discussed in former papers. In this way it can also be ascertained that all weak dibasic acids combine in molecular proportions with isoelectric protein, while strong dibasic acids and diacidic alkalies combine in equivalent proportions with proteins, as is shown by Table I. It follows from this table that for the same pH the amount of HNO_3 , oxalic, and phosphoric acids in com-

bination with the same quantity of originally isoelectric gelatin is always in the proportion of 1:2:3.

We can therefore state that the ratios in which ions combine with proteins are identical with the ratios in which the same ions combine with crystalloids. Or in other words, the forces by which gelatin and egg albumin (and probably proteins in general) combine with acids or alkalies are the purely chemical forces of primary valency.

v.

The most important fact for our purpose is that from the combining ratios just mentioned the influence of acids and bases on the physical properties of proteins can be predicted. This influence is altogether different from that stated in the so-called Hofmeister series of ions or by the ion series of Pauli and his collaborators, and this difference is due to the fact that these latter authors compared the effects of equal quantities of acids or alkalies while we found it necessary to compare the physical properties of solutions of proteins of the same hydrogen ion concentration. If this is done the following rule is found. All those acids whose anion combines as a monovalent ion raise the osmotic pressure, viscosity, swelling of protein about twice as much as the acids whose anion combines as a bivalent anion for the same pH. The same valency rule holds for the cations of different alkalies.

We have seen that at the same pH three times as many c.c. of 0.1 N H_3PO_4 as of HNO_3 are in combination with 1 gm. of originally isoelectric gelatin in 100 c.c. of solution. It follows from this that the anion of gelatin phosphate is the monovalent ion H_2PO_4 and not the trivalent anion PO_4 . It follows likewise from the combining ratios discussed that the anion of oxalic acid in combination with protein is the monovalent anion HC_2O_4 . The same is true for all weak dibasic or tribasic acids, namely that they combine with proteins forming protein salts with monovalent anion. It follows also from the combining

TABLE I

C.c. of 0.01 N Acid in Combination with 10 c.c. of a 1 Per Cent. Gelatin Solution at Different pH

pH	3.1	3.2	3.3	3.4	3.5	3.7	3.9	4.1	4.2	4.3
HNO_3 . . .	4.35	4.1	3.6	3.2	2.85	2.45	1.9	1.45		0.75
Oxalic acid	9.6	8.75	7.6	6.7	6.00	4.3	3.0		1.65	
H_3PO_4 . . .		12.4	10.4	9.8	9.00	7.4	5.8	4.5	2.6	2.1

ratios that the salt of a protein with a strong dibasic acid, as H_2SO_4 , however, must have a divalent anion, *e. g.*, SO_4 . If we compare the viscosity or osmotic pressure of 1 per cent. solutions of originally isoelectric gelatin with different acids of the same pH we find that these properties are identical for all gelatin salts with monovalent anion; in other words, 1 per cent. solutions of gelatin chloride, bromide, nitrate, tartrate, succinate, citrate, or phosphate have all the same vis-

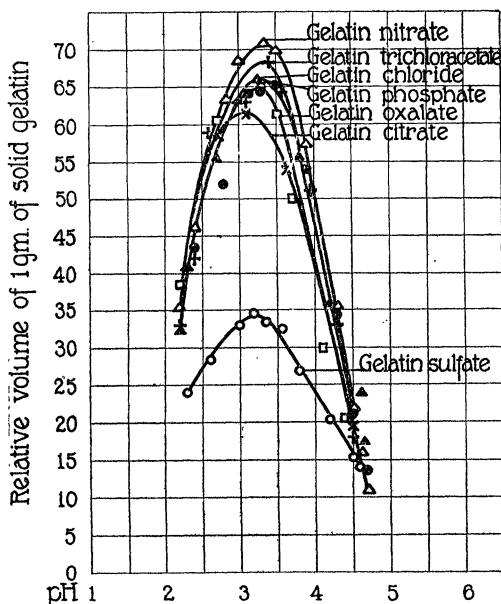


FIG. 2. Influence of different acids upon the swelling of gelatin when plotted over pH as abscissæ. The curves show that nitric, trichloroacetic, hydrochloric, phosphoric, oxalic, and citric acids cause approximately the same degree of swelling, while sulfuric acid causes only about one half the amount of swelling. In the case of gelatin sulfate the anion is divalent; in the case of the other acids used it is monovalent. According to the Hofmeister series the curves for phosphate, oxalate and citrate should coincide with that of sulfate instead of coinciding with that of chloride.

cosity, and the same osmotic pressure at the same pH. The same is true for the swelling (Fig. 2). If we plot the curves for these three properties with pH as abscissæ and the values for osmotic pressure, viscosity, and

swelling as ordinates, we get practically identical curves for gelatin chloride, bromide, nitrate, tartrate, succinate, citrate, and phosphate. The values for swelling are a minimum at pH 4.7 (the isoelectric point of gelatin) they rise rapidly with the fall of pH until they reach a maximum at pH about 3.2, and then they drop again. Each curve is the expression of an individual experiment. The maximum in the curves for gelatin chloride, bromide, nitrate, tartrate, succinate, citrate and phosphate is practically identical, the variations between the values for these acids lying within the limit of variation which we may expect if we plot six different experiments with the same acid. When, however, we plot the same curves for gelatin sulfate, we get curves which are considerably lower, reaching a height of only one half (or a little less than) those of gelatin-acid salts with monovalent anions. It may be of interest to compare our curves with those expected on the basis of Pauli's and Hofmeister's ion series. According to the latter theory the curves for phosphates, oxalates, citrates, and tartrates should be in the region of the SO_4 curve but not in the region of the Cl curve. Those authors who observed such differences did not measure the hydrogen ion concentration, attributing the effects due to the difference in the hydrogen ion concentration of their gelatin solutions erroneously to a difference in the anion effect. These elementary errors form the basis of a number of speculations current in biology and pathology.

When we compare monobasic acids of different strength, *e. g.*, acetic, mono-, di-, and trichloroacetic acids, we find that the weaker the acid the more acid must be contained in a 1 per cent. solution of originally isoelectric gelatin to bring it to the same pH. If we compare the effect of these four acids on the osmotic pressure of gelatin we find that it is (within the limits of accuracy of these experiments) identical for the same pH. The curves for the influence of these four acids on the osmotic pressure of gelatin solution are practically identical when plotted over the pH as abscissæ; and, moreover, the curves

are identical with the curves for HCl or H_3PO_4 in Fig. 1. The explanation of this fact is that at the same pH the same mass of originally isoelectric gelatin is in combination with the same quantity of these four acids and since the anions of these four acids are all monovalent the curves must be identical.

As far as the alkalies are concerned, we notice that the curve representing the effect of the weak base NH_4OH on the physical properties of proteins is the same as that for the strong bases LiOH, NaOH, KOH when plotted over pH as abscissæ, while the curves representing the effect of $\text{Ca}(\text{OH})_2$ or $\text{Ba}(\text{OH})_2$ on the same properties are considerably lower.

It is obvious that the valency of the ion in combination with the protein has a noticeable influence on the properties of the protein salt formed, while the protein salts with ions of the same valency have all the same properties. The fact of the greatest importance is, however, that the influence of acids and bases on the physical properties of proteins is the expression of the combining ratios of the acids or bases with proteins so that we are able to predict the value of the physical properties from the combining ratios. This fact seems to give a final decision in favor of a purely chemical theory of these influences and against the colloidal theories as based on the Hofmeister or Pauli ion series.

The behavior of the proteins therefore contradicts the idea that the chemistry of colloids differs from the chemistry of crystalloids.

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THE AFRICAN RIFT VALLEYS

A RECENT article¹ with the above title by Professor J. W. Gregory, of Glasgow, is of interest from the general summary that it

¹ "The African Rift Valleys," by J. W. Gregory, *Geogr. Jour.*, LVI., 1920, 13-47, with 6 maps, 7 profiles and 8 half-tone plates, and a bibliography of 65 titles.

presents of a remarkable group of natural features, as well as from the ingenious flight of geological imagination by which it explains them. The term, rift valley, introduced by Gregory in connection with his African studies of 25 years ago, designates a longitudinal depression "caused by the material sinking in mass, so that what is now its floor formerly stood level with the highlands on each side." Such valleys therefore contrast strongly with "ordinary valleys, which are caused by the removal piecemeal by rivers or wind of the material that once filled them." The omission of glaciers from the last clause is doubtless prompted by Gregory's disbelief in their capacity to erode.

His article opens with a general and for the most part an empirical account of the "Great Rift Valley" of Africa, and of the volcanoes that occur along it, with little attention to the structure of the region traversed, and with still less attention either to the erosion that the region had suffered before the assumed rifting or to the erosion that the enclosing scarps have suffered since the rifting. Apart from briefly cited opinions of various authors, form alone in most cases is appealed to in evidence of down-faulting. As a result the reader may not feel convinced that all the depressions described as rift valleys really belong in that class. Some of the limiting scarps may be purely cliffs of erosion. Indeed, the inclusion of the Red Sea, with the narrow Gulfs of Suez and of Akaba at its northwest end and the broad Gulf of Aden to the southeast of it, as rift valleys, and the drawing of several "diagonal tectonic lines" along certain parts of the east African coast that are supposed to have been "cut off by . . . faulting" suggest so open a hospitality to the occurrence of rifts and rift valleys as to make the reader wonder whether they are not overworked. The first part of the article is therefore chiefly valuable as a topographic summary of a remarkable region. A critical discussion of the evidence for rifting, based on a review of the many articles cited in the bibliography, would make an excellent subject for an advanced student in physiography.